638. *Studies in Relation to Biosynthesis. Part XX.** *The Structure and Biosynthesis of Curvularin.*

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The mould metabolite, curvularin, has been shown to be a lactone (VI; R = H) containing a twelve-membered ring. Its biosynthesis involves the head-to-tail linkage of eight acetic acid units.

PRELIMINARY work [1,2] indicated the partial structure (I; $R^1 = R^2 = H$, $R^3 = O$) for curvularin, $C_{16}H_{20}O_5$, a metabolite of a *Curvularia* species [F334 in the Nobel Division Collection (Imperial Chemical Industries Limited); Commonwealth Mycological Institute Cat. No. I.M.I. 52,980]. We present here evidence defining the structure and the biosynthetic units involved.

Structure.—The structural work can be resolved into determination of the number of *C*-Me groups, definition of the unknown oxygen functions, and their location.

The original assignment of 2 *C*-Me groups rested on the production of *ca.* 1·5 mols. of acetic acid on Kuhn–Roth oxidation. In our hands this oxidation gave 1·05 and 0·89 mol. in replicate determinations, indicating that curvularin may have only one *C*-Me group. We reached a decision in a novel way. The partial structure (I) suggested biosynthesis, at least in part, from acetic acid units: in particular, formation of the acylresorcinol fragment could be envisaged as involving cyclisation of an intermediate of partial structure (II) containing a carbon chain formed by serial head-to-tail linkage of acetic acid units.[3] We found that the mould incorporated radioactivity from $Me\cdot{}^{14}CO_2H$ into curvularin to the extent of *ca.* 4% and that the acetic acid produced by Kuhn–Roth oxidation contained approximately one-eighth of the total activity, all of it on the carboxyl group. We can therefore infer that the C_{16} metabolite is derived from eight acetic acid units very probably incorporated through a straight-chain precursor, a conclusion later supported by examination of other degradations set out below. It therefore contains one *C*-Me group which is presumably associated with the acetic acid unit at one end of the chain. We believed that the presence of 2 *C*-Me groups would have implied a biosynthesis from eight acetic acid units (one of them decarboxylated), with the

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* Part XIX, Birch, Ryan, and Smith, *J.*, 1958, 4773.

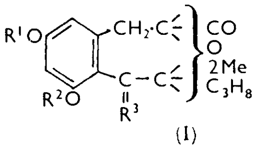
[1] Musgrave, *J.*, 1956, 4301.

[2] *Idem, J.*, 1957, 1104.

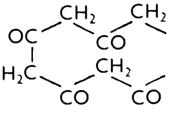
[3] Cf. Birch, Massy-Westropp, and Moye, *Austral. J. Chem.*, 1958, **8**, 539; Birch, Massy-Westropp, Rickards, and Smith. *Proc. Chem. Soc.*, 1957, 98; *J.*, 1958, 360.

introduction of a second methyl group on to a carbon atom in the skeleton derived from the methyl-carbon of acetic acid *e.g.*, as in the expression (III).[4] Kuhn–Roth oxidation of the labelled metabolite would then have resulted in dilution of the active acetic acid from the end acetate unit with inactive acid produced from the methylated position, and the resulting acetic acid would have contained a smaller fraction of the total activity than that found (one-fourteenth of the total if each *C*-Me had contributed equally to the Kuhn–Roth acid). Transmethylations on to carbon [4,5] have been diagnosed in a number of biological systems by location of the radioactivity from $H\cdot{}^{14}CO_2H$ or $[{}^{14}CH_3]$methionine on the, introduced groups. With curvularin radioactivity from $H\cdot{}^{14}CO_2H$ was incorporated, but only to a very small extent and with general distribution throughout the molecule. The absence of transmethylation in the biosynthesis and therefore of a second methyl group in the metabolite is thus confirmed. It is notable that in the acetic acid obtained by Kuhn–Roth oxidation of $H\cdot CO_2H$-derived curvularin the ratio of the labelling on the methyl- and the carboxyl-carbon atoms is approximately 5 : 1.

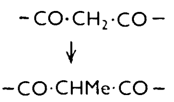
We turn next to the definition of the five oxygen functions. Musgrave [1,2] had proved that two oxygen atoms are associated with phenolic hydroxyl groups and had obtained spectroscopic evidence that two more are associated with carbonyl groups, one of which is ketonic and conjugated with the aromatic nucleus. The nature of the second carbonyl group and of the remaining oxygen atom remained undecided. We confirmed the presence of a ketonic group by formation of a monoxime and located the remaining two oxygen atoms in a lactone group by the following evidence. Curvularin contains no olefinic unsaturation [1] and from the molecular formula must contain at least one ring in addition to the aromatic nucleus. The infrared absorption spectra of curvularin and its dimethyl ether (I; $R^1 = R^2 = Me, R^3 = O$) are consistent with the presence of a lactone group, and both substances react with aqueous alkali but fail to give a positive reaction in the hydroxamic acid test for the carboxylic ester group.[1,2] With warm aqueous potassium hydroxide *in vacuo* curvularin gave an amorphous product which rapidly darkened in alkaline solution in air. Under parallel conditions, but in aqueous-methanolic potassium hydroxide, curvularin dimethyl ether gave an isomer, $C_{18}H_{24}O_5$, containing an acylatable alcoholic hydroxyl group but lacking the carboxyl group expected from hydrolysis of a lactone. We return to the structure of this substance below. By the same treatment, deoxydihydromonomethylcurvularin (I; $R^1 = H$ or $Me, R^2 = Me$ or $H, R^3 = H_2$), formed from deoxydihydrocurvularin [2] (I; $R^1 = R^2 = H, R^3 = H_2$) by treatment with an excess of ethereal diazomethane, gave a hydroxy-carboxylic acid, $C_{17}H_{26}O_5$, revealing the lactone group in this substance and hence in curvularin. Curvularin thus contains an oxo-lactone ring fused in the 4,5-position to a resorcinol nucleus.



(I)



(II)



$$\begin{array}{c}-CO\cdot CH_2\cdot CO-\\ \downarrow \\ -CO\cdot CHMe\cdot CO-\end{array}$$

(III)

Refluxing curvularin with red phosphorus and hydriodic acid in acetic acid gave a neutral product and an acidic fraction containing an alkanoic and an iodoalkanoic acid which were soluble in aqueous sodium hydrogen carbonate and sodium carbonate respectively. The alkanoic acid was identified as octanoic acid by its paper-chromatographic behaviour and infrared spectrum. The iodo-acid was shown to be an iodo-octanoic acid by reduction with Raney alloy and sodium hydroxide [6] to octanoic acid. The neutral fraction had infrared bands at 1780 and 1181 cm.$^{-1}$, suggesting the presence of a γ-lactone.

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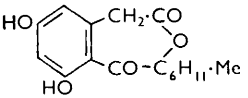
[4] Cf. Birch, Fitton, Pride, Ryan, Smith, and Whalley, *J.*, 1958, 4576.

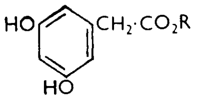
[5] Alexander and Schwenk, *J. Amer. Chem. Soc.*, 1957, **79**, 4554; Alexander, Gold, and Schwenk, *J. Biol. Chem.*, 1958, **232**, 599.

[6] Schwenk, Papa, Whitman, and Ginsberg, *J. Org. Chem.*, 1944, **9**, 1.

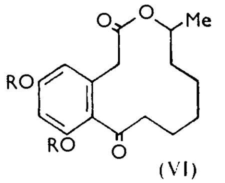
Controlled oxidation by chromic acid[7] gave acetic, propionic, butyric, and succinic acid. Fusion with potassium hydroxide gave propionic, valeric, and a trace of acetic acid. The formation of succinic acid by oxidation and valeric acid by alkali fusion, together with the occurrence of octanoic acids as fission products, strongly suggests that the neutral product is the lactone of 4-hydroxyoctanoic acid. The formation of octanoic acid derivatives is most readily interpreted as due to acid-hydrolysis of a 4-acylresorcinol system; the partial structure may accordingly be expanded to (IV). This interpretation was supported by fission under non-reducing conditions with hydrobromic acid in acetic acid. The products included a bromo-acid, converted by reductive dehalogenation into octanoic acid, the above-mentioned lactone, and a phenolic carboxylic acid. The phenolic acid was difficult to purify and was converted into its methyl ester which was identified as methyl 3,5-dihydroxyphenylacetate[8] (V; R = Me). It is notable that the isolation of two C_8 fragments of apparently mutually exclusive structures accounts for all of the carbon atoms of curvularin. Together with the formation of 2-carboxymethyl-4,6-dimethoxyphenylglyoxylic acid on oxidation of dimethyl curvularin by alkaline permananate, these results prove the carbon skeleton of structure (IV) to be correct. The structural feature still undefined is the point of lactonisation on the octanoyl chain. No significance can be attached to the formation of a γ-lactone since the forcing conditions used would be expected to give the most stable lactone from the halogeno-, hydroxy-, or acetoxy-octanoic acid produced in the initial fission. The location of the lactonic-oxygen atom at the 7-position of the chain follows from the formation of acetic acid as the only steam-volatile acid by controlled oxidation of curvularin by chromic acid,[7] and of iodoform and pimelic acid on oxidation of an alkaline solution of curvularin with hydrogen peroxide and treatment of the product with sodium hypoiodite. Curvularin accordingly has structure (VI; R = H).



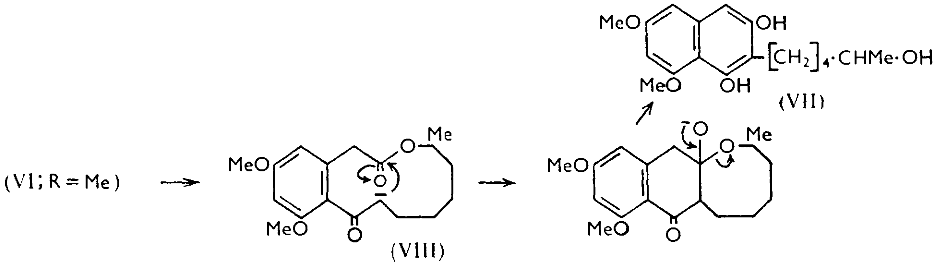
(IV)



(V)



(VI)



(VII)

(VIII)

This structure is consistent with the spectral and chemical properties. The ultraviolet and infrared absorption of curvularin indicate a lower degree of conjugation of the carbonyl group with the aromatic nucleus than is observed with the analogous 2,4-dihydroxy-6-methylacetophenone, presumably because of non-coplanarity.[2] In 2,4-dihydroxy-6-methylacetophenone coplanarity is attributed to hydrogen-bonding between the carbonyl and the 2-hydroxyl group which apparently outweighs the tendency of the *o*-alkyl group to force the carbonyl group out of the plane of the aromatic nucleus (for a discussion see ref. 2). In curvularin the same two effects may operate but coplanarity is probably resisted by the high energy of the requisite conformations of the macrocycle. Leonard and

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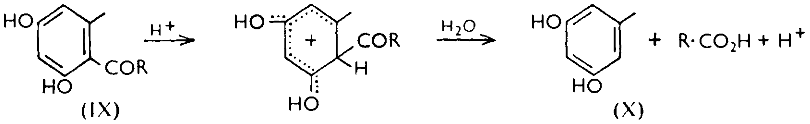
[7] Garbers, Schmid, and Karrer, *Helv. Chim. Acta*, 1954, **37**, 1336.
[8] Theilacher and Schmid, *Annalen*, 1950, **570**, 18.

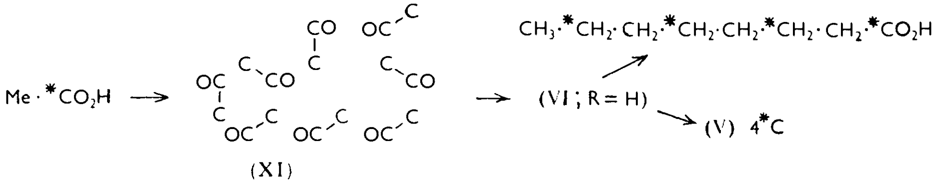
Owens [9] have recently shown, chemically and spectroscopically, the resistance to coplanarity of the C=C–C=O system in cyclododec-2-enone.

The nature of the isomer $C_{18}H_{24}O_5$ obtained from di-*O*-methylcurvularin with methanolic alkali follows directly from the structure (VI; R = Me). The infrared spectrum indicates only phenolic and alcoholic hydroxyl groups; the ultraviolet absorption is reminiscent of a hydroxynaphthalene system, and the compound is reasonably formulated as the naphthol (VII) produced by the transannular Claisen-condensation represented by the sequence (VI; R = Me) ⟶ (VIII) ⟶ (VII). With ethereal diazomethane this naphthol afforded only a trimethoxynaphthalene and it resisted complete esterification with acetic anhydride; this we attribute to the low reactivity of the *peri*-hydroxyl group. The formation of naphthols of type (VII) explains the failure of the hydroxamic acid test for the carboxylic ester group on curvularin and di-*O*-methylcurvularin.



Removal of the acyl side chain under strongly acid conditions may plausibly be regarded as the reverse of a Friedel–Crafts acylation, in which the initial protonation in the sequence (IX) ⟶ (X) would be facilitated by delocalisation of the positive charge on the hydroxylic oxygen atoms. This reaction is apparently analogous to the formation of resorcinol (as shown by production of fluorescein) from 2,4-dihydroxyacetophenone by sulphuric acid.[10]

Biosynthesis.—Evidence for biosynthesis involving head-to-tail linkage of eight acetate units is given above. Confirmation is provided by the fission of the labelled curvularin by hydrogen bromide. The two C_8 fragments obtained from this reaction, each representing four intact acetate units, contained, as required, equal amounts of radioactivity which together accounted for the total. The biosynthesis may be represented as (XI) ⟶ (VI; R = H). Curvularin is the first example of a naturally occurring macrocyclic lactone having a methyl group as the only alkyl substituent, and constitutes an interesting structural (and possibly biosynthetic) link between the macrolide antibiotics [11] (*e.g.*, methymycin [11b]) and the acetate-derived phenols.[12] The remarkable ease of the transannular cyclisation, giving the naphthol (VII), prompts the speculation that macrocyclic lactones may in some cases be intermediates in the production of polycyclic aromatic acetate-derived compounds.



Experimental

Ultraviolet spectra were taken in ethanol. Light petroleum was of b. p. 40—60°. Carboxylic acids were chromatographed as the ammonium salts, by ascending development on Whatman No. 1 paper. Solvent systems were butanol–1·5N-aqueous ammonia [13] and ethanol–

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[9] Leonard and Owens, *J. Amer. Chem. Soc.*, 1958, **80**, 6039.

[10] Torrey and Brewster, *J. Amer. Chem. Soc.*, 1908, **30**, 862.

[11] (*a*) Woodward, *Angew. Chem.*, 1957, **69**, 50; (*b*) Djerassi and Zderic, *J. Amer. Chem. Soc.*, 1956, **78**, 2907.

[12] Birch and Smith, Chem. Soc. Special Publ. No. 12, 1958, p. 1.

[13] Reid and Lederer, *Biochem. J.*, 1951, **50**, 60.

aqueous ammonia [14] for mono- and di-carboxylic acids respectively. General instructions for radioactive assay are as for Part XVII.[4]

Curvularin.—*Curvularia* was cultured as previously described [1] except that the glucose concentration was doubled. The crude metabolite was purified by percolation of its ethyl acetate solution through a short column of the earth " Florisil " (Floridin Co., Warren, Pennsylvania) and recrystallisation from chloroform–ether or benzene–methanol. Curvularin was obtained as plates, m. p. 206—207° (Found: *C*-Me, 5·4, 4·6. Calc. for $C_{16}H_{20}O_5$: 1*C*-Me, 5·15%) and had $\nu_{max.}$ (in Nujol) 3290, 1700, 1660, and 1610 cm.$^{-1}$. Curvularin (150 mg.) was heated on the steam-bath under nitrogen in pyridine (5 c.c.) with hydroxylamine hydrochloride (110 mg.) for 2 hr. The gummy product (153 mg.) in ether was filtered through a short " Florisil " column. Crystallisation from benzene–ether gave curvularin oxime as the benzene *solvate*, m. p. 188—190° (Found: C, 68·1; H, 6·95; N, 3·8. $C_{16}H_{21}O_5N,C_6H_6$ requires C, 68·5; H, 7·1; N, 3·6%), $\lambda_{max.}$ 288, $\lambda_{infl.}$ 217 and 248 mμ (log ε 3·40, 4·26, and 3·68 respectively), $\nu_{max.}$ (in Nujol) 3310, 1702, 1630, and 1600 cm.$^{-1}$.

2-5′-*Hydroxyhexyl*-6,8-*dimethoxynaphthalene*-1,3-*diol* (VII).—Di-*O*-methylcurvularin (490 mg.) in 5% methanolic potassium hydroxide (8 c.c.) was heated at 14 mm. for 20 min. on the water-bath. The cooled solution was acidified with 2N-sulphuric acid and left at 0° overnight. The precipitate was repeatedly recrystallised from benzene with " Florisil " as decolorising agent, to give the *naphthol* (VII), m. p. 103—105° (Found: C, 67·9; H, 7·75; OMe, 18·9. $C_{18}H_{24}O_5$ requires C, 67·5; H, 7·55; 2OMe, 19·4%), $\lambda_{max.}$ 244, 291, 294, 302, and 329 (infl.) mμ (log ε 4·89, 3·64, 3·64, 3·65, and 3·40) (Schmid and his co-workers [15] give similar data for related hydroxy- and methoxy-methylnaphthalenes), $\nu_{max.}$ (in Nujol) 3434, 3140, and 1636 cm.$^{-1}$ (in carbon tetrachloride) 3602, 3413, and 1639 cm.$^{-1}$. The naphthol gave a red ferric test, and with acetic anhydride in pyridine for two days gave an alkali-insoluble oil, $\lambda_{max.}$ 237, 288, 322, and 336 mμ (log ε 4·66, 3·82, 3·59, and 3·56), $\lambda_{infl.}$ 262 and 298 mμ (log ε 3·91 and 3·76), $\nu_{max.}$ (in carbon disulphide) 3410, 1763, 1731, 1635, 1250, and 1210 cm.$^{-1}$. The product from the naphthol and an excess of ethereal diazomethane was repeatedly recrystallised from benzene–light petroleum (with " Florisil "), to give the *trimethoxynaphthalene derivative*, m. p. 80—83° (Found: C, 68·4; H, 7·7; OMe, 27·6. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8; 3OMe, 27·8%), $\lambda_{max.}$ 246, 290, 314, and 329 mμ (log ε 4·91, 3·75, 3·52, and 3·45), $\lambda_{infl.}$ 297 mμ (log ε 3·68), $\nu_{max.}$ (in carbon disulphide) 3597, 3410, and 1631 cm.$^{-1}$, insoluble in alkali and forming a dark red *picrate* (from benzene), m. p. 134—135° (Found: C, 53·6; H, 5·15; N, 7·5; OMe, 16·4. $C_{25}H_{29}O_{12}N_3$ requires C, 53·3; H, 5·2; N, 7·5; OMe, 16·5%].

Curvularin, treated with 5% aqueous potassium hydroxide as for dimethylcurvularin, gave an amorphous solid, $\nu_{max.}$ (in Nujol) 3360 and 1637 cm.$^{-1}$, which could not be purified owing to very rapid darkening of the solutions in air.

Deoxydihydro-O-*methylcurvularin.*—The product from deoxydihydrocurvularin [2] (730 mg.) and excess of diazomethane in ether–methanol, on recrystallisation from chloroform–ether, gave *deoxydihydromethylcurvularin* (400 mg.), m. p. 193—195° (Found: C, 70·3; H, 8·1; OMe, 10·1. $C_{17}H_{24}O_4$ requires C, 69·9; H, 8·3; OMe, 10·6%), $\nu_{max.}$ (in Nujol) 3353 and 1692 cm.$^{-1}$. The methyl ether was insoluble in alkali and was converted by acetic anhydride in pyridine into an oil, $\nu_{max.}$ (in carbon disulphide) 1765, 1730, and 1214 cm.$^{-1}$, from which the methyl ether was recovered after hydrolysis with 10% ethanolic sulphuric acid.

The methyl ether (90 mg.) in 5% methanolic potassium hydroxide (5 c.c.) was warmed under reduced pressure for 20 min. on the steam-bath. The cooled solution was acidified and kept overnight at 0°. The precipitate (70 mg.) was recrystallised from benzene to give the *acid*, m. p. 109—111° (Found: C, 66·0; H, 8·5; OMe, 9·9. $C_{17}H_{26}O_5$ requires C, 65·8; H, 8·4; 1OMe, 10·0%), $\lambda_{max.}$ 285 mμ (log ε 3·48), $\lambda_{infl.}$ 225 mμ (log ε 3·93), $\nu_{max.}$ (in Nujol) 3360, 3180, and 1689 cm.$^{-1}$, soluble in aqueous sodium hydrogen carbonate, and giving no ferric test.

Fission of Curvularin by Hydriodic Acid.—Curvularin (1 g.) was refluxed for 1·3 hr. with red phosphorus (0·5 g.) in acetic acid (15 c.c.) and hydriodic acid (3 c.c.; *d* 1·6). Water was added and the mixture was extracted with ether. The product was divided into fractions which were soluble in aqueous sodium hydrogen carbonate (A), sodium carbonate (B), and sodium hydroxide (negligible), and insoluble in alkali (C). Fraction A, after being washed with water to remove

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[14] Cheftel, Munier, and Macheboeuf, *Bull. Soc. Chim. biol.*, 1952, **34**, 380.

[15] Schmid and Burger, *Helv. Chim. Acta*, 1952, **35**, 931; Ebnöther, Meijer, and Schmid, *ibid.*, 1952, **35**, 911.

acetic acid, was steam-distilled and the product collected with light petroleum. It was obtained as an oil (24 mg.), identified as octanoic acid by the infrared absorption and behaviour on a paper chromatogram. Fraction B (600 mg.), the iodo-acid, was distilled [b. p. 100—110° (bath)/1 mm.], and a portion was converted into the lithium salt, which with *p*-bromophenacyl bromide in hot aqueous ethanol (2 hr.) gave *p*-bromophenacyl iodide, m. p. 97—99°, identical with authentic material prepared by Collet's method.[16]

Raney alloy (800 mg.) was added portionwise with stirring to the iodo-acid (220 mg.) in 5% aqueous sodium hydroxide (25 c.c.). When the reaction had moderated the mixture was heated on the steam-bath for 1 hr. The nickel was removed and the solution poured into 10N-hydrochloric acid. The product was identified as octanoic acid on the basis of the infrared absorption spectrum and paper-chromatographic behaviour. The *p*-bromophenacyl ester, m. p. 65—67°, was identical with authentic material.

Fraction C (14 mg.) had an odour of coconut, and $\nu_{\max.}$ (in carbon disulphide) 1780, 1385, and 1181 cm.$^{-1}$ (γ-lactone). Chromic acid oxidised [7] this lactone (4 mg.) to (*a*) a mixture of steam-volatile acids obtained as the ammonium salts and identified by paper chromatography as acetic, propionic and butyric acid (R_F 0·10, 0·20, and 0·29 respectively), and (*b*) an acid, not volatile in steam, identified by paper chromatography as succinic (R_F 0·23). The lactone (10 mg.) was heated under nitrogen with powdered potassium hydroxide (80 mg.) to 380° for 10 min. and kept thereat for a further 15 min. Water was added to the cooled residue, and the acidified solution was steam-distilled. The distillate was neutralised with ammonia and concentrated. Paper chromatography showed the presence of ammonium acetate (trace), propionate and valerate (R_F 0·10, 0·19, and 0·45).

Fission of Curvularin by Hydrobromic Acid.—Curvularin (800 mg.) was refluxed in acetic acid (8 c.c.) and hydrobromic acid (6 c.c.) for 1·3 hr. Water was added, and the solution was saturated with salt and extracted with ether. The product was separated into fractions A, B, and C as in the fission by hydriodic acid. Acidification of the aqueous alkaline solution of fraction A, followed by extraction with light petroleum, gave a bromine-containing oil (300 mg.) which was reductively dehalogenated as for the iodo-acid above, giving octanoic acid identified by the infrared spectrum, paper chromatography, and conversion into the *p*-bromophenacyl ester. The residual aqueous solution was saturated with salt and extracted with ether. Extraction of the resulting red tar (300 mg.) with boiling benzene gave cream crystals, m. p. 124—126°, difficult to purify further, which gave a violet ferric test. These were kept overnight in methanol previously saturated with dry hydrogen chloride. The product was sublimed at 120—130° (bath)/0·05 mm. and recrystallised from benzene, to give methyl 3,5-dihydroxyphenylacetate, m. p. 109—111° undepressed by a synthetic sample [8] (Found: C, 59·6; H, 5·55. Calc. for $C_9H_{10}O_4$: C, 59·3; H, 5·5%). The infrared spectra of the samples were identical.

Fraction B (120 mg.) contained more of the above bromine-containing acid. Fraction C (60 mg.) contained the γ-lactone isolated from the hydriodic acid fission.

Oxidation of Curvularin.—(i) Chromic acid oxidised [7] curvularin (20 mg.) to acetic acid as the only steam-volatile acid, identified by paper chromatography.

(ii) To curvularin (50 mg.) in 0·1N-aqueous sodium hydroxide (6 c.c.) was added 30% hydrogen peroxide (3 c.c.). After 4 hr. excess of oxidant was destroyed with 5% palladium–charcoal (10 mg.) in the presence of a trace of ferric chloride. The catalyst was removed and aqueous sodium hypoiodite was added. After 30 min. at room temperature the solution was acidifed and excess of oxidant was destroyed with sodium hydrogen sulphite. Continuous ether-extraction gave a product containing two (probably monocarboxylic) acids (R_F in ethanol–aqueous ammonia 0·52 and 0·66). Reoxidation with aqueous sodium hypoiodite gave iodoform, m. p. 120° after sublimation at 110°/10 mm., and an acid fraction containing pimelic acid (R_F 0·35). Recrystallisation of this fraction from benzene–light petroleum gave pimelic acid, m. p. 102—104° undepressed by an authentic specimen of m. p. 104—106°.

(iii) Potassium permanganate (300 mg.) was added with stirring to curvularin (50 mg.) in 3% aqueous sodium hydroxide (10 c.c.) at 0°. Further oxidant (200 mg.) was added portionwise with stirring during 1 hr., and the mixture was then warmed at 60° for 3 hr. Sodium hydrogen sulphite was added to the cooled acidified mixture, and aqueous sodium hypoiodite was added to the resulting clear solution. After 40 min. the solution was worked up as under (ii), to give a mixture containing succinic, glutaric, adipic and pimelic (trace) acid (R_F 0·18, 0·22, 0·27, and 0·31 respectively).

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[16] Collet, *Bull. Soc. chim. France*, 1900, **23**, 830.

Oxidation of deoxydihydrocurvularin as under (i) and (ii) above gave, respectively, acetic acid and a mixture of succinic, glutaric, adipic, and pimelic acid.

Degradation of [^{14}C]*Curvularin.*—Relative molar activities (*a*) and (*b*) below refer to the labelled metabolite and its degradation products derived from $CH_3{\cdot}^{14}CO_2Na$ and $H{\cdot}^{14}CO_2Na$ respectively. Theoretical numbers of labelled carbon atoms in substances of the $CH_3{\cdot}^{14}CO_2Na$ series are based on the distribution given by head-to-tail linkage of eight acetate residues.

Curvularia was grown as above except that after 8 days aqueous $CH_3{\cdot}^{14}CO_2Na$ (0·2 mc) was distributed between two flasks (1·5 l. of medium). The crude curvularin (0·54 g.) isolated after a further 11 days' growth was combined with material obtained by carrier-extraction of the residual culture fluid with pure unlabelled curvularin (total 450 mg.) and then diluted with further unlabelled material. Recrystallisation gave pure labelled curvularin (9·02 g., 7·5 μc, 3·8%) [Found: 10^{-3} r.m.a., (*a*) 322].

The mould was grown as above, aqueous $H{\cdot}^{14}CO_2Na$ (50 μc) being substituted for $CH_3{\cdot}^{14}CO_2Na$. Medium from 2 culture flasks (1·5 l.) gave [^{14}C]curvularin (0·38 g.; 0·08 μc, 0·16%) [Found: 10^{-3} r.m.a., (*b*) 80·9].

Kuhn–Roth oxidation gave acetic acid, collected as lithium acetate [Found: 10^{-3} r.m.a., $BaCO_3$ (Me), (*a*) 0, (*b*) 9·08; $BaCO_3(CO_2H)$, (*a*) 43·4, (*b*) 1·78. 1*C requires (*a*) 40·3] and converted into the *p*-bromophenacyl ester, m. p. 86° [Found: 10^{-3} r.m.a., (*a*) 42·5, (*b*) 10·8 1*C requires (*a*) 40·3].

Hydrobromic acid fission gave methyl 3,5-dihydroxyphenylacetate [Found: 10^{-3} r.m.a., (*a*) 160. 4*C requires (*a*) 161] and octanoic acid converted into the *p*-bromophenacyl ester [Found: 10^{-3} r.m.a., 163. 4*C requires (*a*) 161].

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